

APPENDIX D: CLEAN COPY OF AMENDED CLAIMS

WE CLAIM:

1. (Amended) An *in vitro* adhesion cell culture comprising at least 90% GFAP⁺ cells, wherein
 - a) one or more cells in the culture have the capacity to differentiate into neurons;
 - b) the cell culture divides in a culture medium containing serum and at least one proliferation-inducing growth factor; and
 - c) one or more cells in the culture differentiate into neurons upon withdrawal of both serum and the proliferation-inducing growth factor.
2. (Amended) The cell culture of claim 1, wherein greater than 50% of cells in the culture are nestin⁺ under proliferation-promoting culture conditions.
3. (Amended) An *in vitro* cell culture consisting essentially of:
 - (a) a culture medium containing serum and at least one proliferation-inducing growth factor; and
 - (b) cells derived from the central nervous system of a mammal, wherein:
 - (i) at least 90 % of the cells are glial fibrillary acidic protein immunoreactive (GFAP⁺),
 - (ii) the cells are capable proliferating in a culture medium containing serum and at least one proliferation-inducing growth factor, and
 - (iii) the cells are capable of differentiating into a population of at least 10% neurons in the absence of both the serum and the proliferation-inducing growth factor from the culture medium.
4. (Amended) The cell culture of claim 3, wherein greater than 50% of cells in the culture are nestin immunoreactive (i.e., nestin⁺) under proliferation-promoting culture conditions.
5. (Amended) The cell culture of claim 1 wherein the cell culture differentiates into at least

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10% neurons under differentiation-inducing culture conditions.

6. The cell culture of claim 1 or 3, wherein the cell culture differentiates into at least 25% neurons under differentiation-inducing culture conditions.
7. The cell culture of claim 1 or 3, wherein, under differentiation-inducing culture conditions, greater than 50% of differentiated neuronal cells have a GABA-ergic phenotype.
8. The cell culture of claim 1 or 3, wherein the culture is capable of at least 6 doublings.

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9. (Amended) The cell culture of claim 1 or 3, wherein the culture is capable of at least 12 doublings.

10. The cell culture of claim 1 or 3, wherein the culture is capable of at least 18 doublings.
11. The cell culture of claim 1 or 3, wherein the cells are derived from the lateral ganglionic eminence (LGE) or medial ganglionic eminence (MGE) of the mammal.
12. The cell culture of claim 1 or 3, wherein the doubling rate of the culture is faster than seven days.
13. The cell culture of claim 1 or 3, wherein the cells in the culture are murine.
14. The cell culture of claim 1 or 3, wherein the cells in the culture are human.
15. The cell culture of claim 1 or 3, wherein fewer than 5% of the cells in the culture are β -tubulin III immunoreactive (β -tubulin III⁺) under proliferation-promoting culture conditions and between 10-40% of the cells in the culture are β -tubulin III immunoreactive (β -tubulin III⁺)

under differentiation-inducing culture conditions.

16. The cell culture of claim 1 or 3, wherein the proliferation-inducing growth factor is selected from the group consisting of epidermal growth factor, amphiregulin, basic fibroblast growth factor, acidic fibroblast growth factor, transforming growth factor alpha, leukemia inhibitor factor, ciliary neurotrophic factor and combinations thereof.

17. The cell culture of claim 3, wherein the culture is an adhesion culture.

18. (Amended) The cell culture of claim 1 or 3, wherein at least a portion of the cells in culture differentiate into radial glia in the absence of serum from the culture medium.

19. The cell culture of claim 18, wherein the radial glia are both GFAP⁺ and vimentin positive.

20. The cell culture of claim 18, wherein the morphology of the radial glia is:

- (a) bipolar;
- (b) elongated; and
- (c) non-fibrillary.

21. (Amended) The cell culture of claim 1 or 3, wherein at least a portion of the cells in culture, under differentiation-inducing culture conditions, differentiate into neurons that exhibit:

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- (a) axon-dendrite polarity,
 - (b) synaptic terminals, and
 - (c) localization of proteins involved in synaptogenesis and synaptic activity including
 - (i) neurotransmitter receptors,
 - (ii) transporters, and
 - (iii) processing enzymes.

22. A method of producing a neuronal cell *in vitro* comprising the steps of:
- (a) obtaining neural tissue from a mammal, the neural tissue containing at least one GFAP⁺ cell capable of producing progeny that is a GFAP⁺ cell;
 - (b) dissociating the neural tissue to obtain a cell suspension comprising the GFAP⁺ cell;
 - (c) culturing the cell suspension in a first culture medium containing serum and at least one proliferation-inducing growth factor to proliferate said GFAP⁺ cell and produce a GFAP⁺ cell progeny; and
 - (d) differentiating the cell progeny in a second culture medium that is substantially free of both the serum and the proliferation-inducing growth factor.
23. The method of claim 22, wherein the cell is nestin⁺.
24. A method of producing a non-neuronal cell *in vitro* comprising the steps of:
- (a) obtaining neural tissue from a mammal, the neural tissue containing at least one GFAP⁺ cell capable of producing progeny that is a GFAP⁺ cell;
 - (b) dissociating the neural tissue to obtain a cell suspension comprising the GFAP⁺ cell;
 - (c) culturing the cell suspension in a first culture medium containing serum and at least one proliferation-inducing growth factor to proliferate said GFAP⁺ cell and produce a GFAP⁺ cell progeny; and
 - (d) differentiating the cell progeny in a second culture medium that is substantially free of the serum.
25. The method of claim 24, wherein the cell is nestin⁺.
26. The method of claim 24, wherein the neuronal cell is an astrocyte or an oligodendrocyte.
27. A method of producing a non-tumorigenic, genetically modified GFAP⁺ cell comprising

the steps of:

- (a) obtaining neural tissue from a mammal, the neural tissue containing at least one GFAP⁺ cell capable of producing progeny that is a GFAP⁺ cell;
- (b) dissociating the neural tissue to obtain a cell suspension comprising the GFAP⁺ cell;
- (c) culturing the cell suspension in a first culture medium containing serum and at least one proliferation-inducing growth factor to proliferate said GFAP⁺ cell and produce a GFAP⁺ cell progeny; and
- (d) genetically modifying the GFAP⁺ cell to express a biologically active agent.

28. The method of claim 27, wherein the cell is nestin⁺.

29. The method of claim 27, wherein the biologically active agent is selected from the group consisting of growth factors, trophic factors, growth factor receptors, neurotransmitters, neuropeptides, neurotrophic factors, hormones, enzymes, cytokines, lymphokines, anti-angiogenic factors, and antibodies.

30. A method of producing a non-tumorigenic, genetically modified differentiated neural cell culture comprising the steps of:

- (a) obtaining neural tissue from a mammal, the neural tissue containing at least one GFAP⁺ cell capable of producing progeny that is a GFAP positive neural cell;
- (b) dissociating the neural tissue to obtain a cell suspension comprising the GFAP⁺ cell;
- (c) culturing the cell suspension in a first culture medium containing serum and at least one proliferation-inducing growth factor to proliferate said GFAP⁺ cell and produce a GFAP⁺ cell progeny;
- (d) differentiating the cell progeny to contain at least 10% neurons in a second culture medium that is substantially free of both the serum and the proliferation-inducing growth factor.

- (e) genetically modifying the differentiated cell to express a biologically active agent.

31. The method of claim 30, wherein the cell is nestin⁺.

32. A method of producing a non-tumorigenic, genetically modified non-neuronal cell culture comprising the steps of:

- (a) obtaining neural tissue from a mammal, the neural tissue containing at least one GFAP⁺ cell capable of producing progeny that is a GFAP positive neural cell;
- (b) dissociating the neural tissue to obtain a cell suspension comprising the GFAP⁺ cell;
- (c) culturing the cell suspension in a first culture medium containing serum and at least one proliferation-inducing growth factor to proliferate said GFAP⁺ cell and produce a GFAP⁺ cell progeny;
- (d) differentiating the cell culture to contain at least 10% radial glia in a second culture medium that is substantially free of serum, wherein the radial glia are GFAP⁺ and vimentin positive, and
- (e) genetically modifying the non-neuronal cell.

33. The method of claim 32, wherein the cell is nestin⁺.

34. A method of transplanting GFAP⁺ nestin⁺ cell progeny to a host comprising:
- (a) obtaining neural tissue from a mammal, said neural tissue containing at least one GFAP⁺ nestin⁺ cell capable of producing progeny that are capable of differentiating into neurons and glia;
 - (b) dissociating the neural tissue to obtain a cell suspension comprising the GFAP⁺ nestin⁺ cell;
 - (c) culturing the cell suspension in a culture medium containing serum and at least one proliferation-inducing growth factor to proliferate the GFAP⁺ nestin⁺ cell and produce GFAP⁺ nestin⁺ cell progeny; and
 - (d) transplanting the GFAP⁺ nestin⁺ cell progeny to the host.
35. The method of claim 34 wherein prior to step (d), the GFAP⁺ nestin⁺ cell progeny are genetically modified to express a biologically active agent.
36. The method of claim 35 wherein the biologically active agent is selected from the group consisting of growth factors, trophic factors, growth factor receptors, neurotransmitters, neuropeptides, neurotrophic factors, hormones, enzymes, cytokines, lymphokines, anti-angiogenic factors, and antibodies.
37. A method for determining the effect of at least one biological agent on a GFAP⁺ nestin⁺ cell comprising:
- (a) obtaining neural tissue from a mammal, the neural tissue containing at least one GFAP⁺ nestin⁺ cell capable of producing progeny that is a GFAP⁺ nestin⁺ cell;
 - (b) dissociating the neural tissue to obtain a cell suspension comprising the GFAP⁺ nestin⁺ cell;
 - (c) culturing the cell suspension in a first culture medium containing serum and at least one proliferation-inducing growth factor to proliferate said GFAP⁺ nestin⁺ cell and produce a GFAP⁺ nestin⁺ cell progeny;
 - (d) contacting the proliferated GFAP⁺ nestin⁺ cell with the biological agent, and

(e) determining the effect of the biological agent on the GFAP⁺ nestin⁺ cells.

38. The method of claim 37, wherein the biological agent is selected from the group consisting of basic fibroblast growth factor, acid fibroblast growth factor, epidermal growth factor, transforming growth factor α , transforming growth factor β , nerve growth factor, insulin like growth factor, platelet derived growth factor, glia-derived neurotrophic factor, brain derived neurotrophic factor, ciliary neurotrophic factor, phorbol 12-myristate 13-acetate, tryphotin, activin, thyrotropin releasing hormone, interleukins, bone morphogenic protein, macrophage inflammatory proteins, heparan sulfate, amphiregulin, retinoic acid, tumor necrosis factor α , fibroblast growth factor receptor, epidermal growth factor receptor.

39. A method for determining the effect of at least one biological agent on the differentiation of GFAP⁺ nestin⁺ cells comprising:

- (a) obtaining neural tissue from a mammal, the neural tissue containing at least one GFAP⁺ nestin⁺ cell capable of producing progeny that is a GFAP⁺ nestin⁺ cell;
- (b) dissociating the neural tissue to obtain a cell suspension comprising the GFAP⁺ nestin⁺ cell;
- (c) culturing the cell suspension in a first culture medium containing serum and at least one proliferation-inducing growth factor to proliferate said GFAP⁺ nestin⁺ cell and produce a GFAP⁺ nestin⁺ cell progeny;
- (c) inducing the proliferated GFAP⁺ nestin⁺ cells to differentiate in a second culture medium in the presence said biological agent, and
- (d) determining the effects of the biological agent on the differentiation of the GFAP⁺ nestin⁺ cells.

40. A method for determining the effect of at least one biological agent on differentiated GFAP⁺ nestin⁺ cells comprising:
- (a) obtaining neural tissue from a mammal, the neural tissue containing at least one GFAP⁺ nestin⁺ cell capable of producing progeny that is a GFAP⁺ nestin⁺ cell;
 - (b) dissociating the neural tissue to obtain a cell suspension comprising the GFAP⁺ nestin⁺ cell;
 - (c) culturing the cell suspension in a first culture medium containing serum and at least one proliferation-inducing growth factor to proliferate the GFAP⁺ nestin⁺ cell and produce a GFAP⁺ nestin⁺ cell progeny;
 - (d) inducing the proliferated GFAP⁺ nestin⁺ cells to differentiate into neurons or glia,
 - (e) contacting the differentiated neural cells with the biological agent, and
 - (f) determining the effects of the biological agent on said differentiated neural cells.
41. A cDNA library prepared from a GFAP⁺ nestin⁺ cell.
42. A cell population consisting essentially of isolated GFAP⁺ nestin⁺ cells.
43. The method of claim 22 wherein under differentiation-inducing culture conditions, the majority of differentiated neuronal cells have a GABA-ergic phenotype.
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- B4 44. (Amended) The method of claim 22 wherein greater than 50% of differentiated neuronal cells are immunoreactive with striatal neuronal markers.
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45. The method of claim 44 wherein said striatal neuronal markers are DLX1 and/or MEIS2.
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- B5 46. (Amended) The method of claim 22 wherein greater than 50% of differentiated neuronal cells are not immunoreactive with cortical neuronal markers.
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47. The method of claim 46 wherein the cortical neuronal markers is PAX6.

48. (Amended) The method of claim 22 wherein greater than 50% of differentiated neuronal cells are not immunoreactive with neuronal markers of the medial ganglionic eminence.

49. The method of claim 48 wherein one of said neuronal markers of the medial ganglionic eminence is NKX2.1.

50. (Amended) The culture of claim 1 or 3 wherein under differentiation-inducing culture conditions, greater than 50% of differentiated neuronal cells have a GABA-ergic phenotype.

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51. (Amended) The culture of claim 1 or 3 wherein greater than 50% of differentiated neuronal cells are immunoreactive with striatal neuronal markers.

52. The culture of claim 51 wherein said striatal neuronal markers are DLX1 and/or MEIS2.

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53. (Amended) The culture of claim 1 or 3 wherein greater than 50% of differentiated neuronal cells are not immunoreactive with cortical neuronal markers.

54. The culture of claim 53 wherein the cortical neuronal marker is PAX6.

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55. (Amended) The culture of claim 1 or 3 wherein greater than 50% of differentiated neuronal cells are not immunoreactive with neuronal markers of the medial ganglionic eminence.

56. The culture of claim 55 wherein one of said neuronal markers of the medial ganglionic eminence is NKX2.1.